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Mastitis Studies in Tennessee

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MASTITIS STUDIES IN TENNESSEE

By
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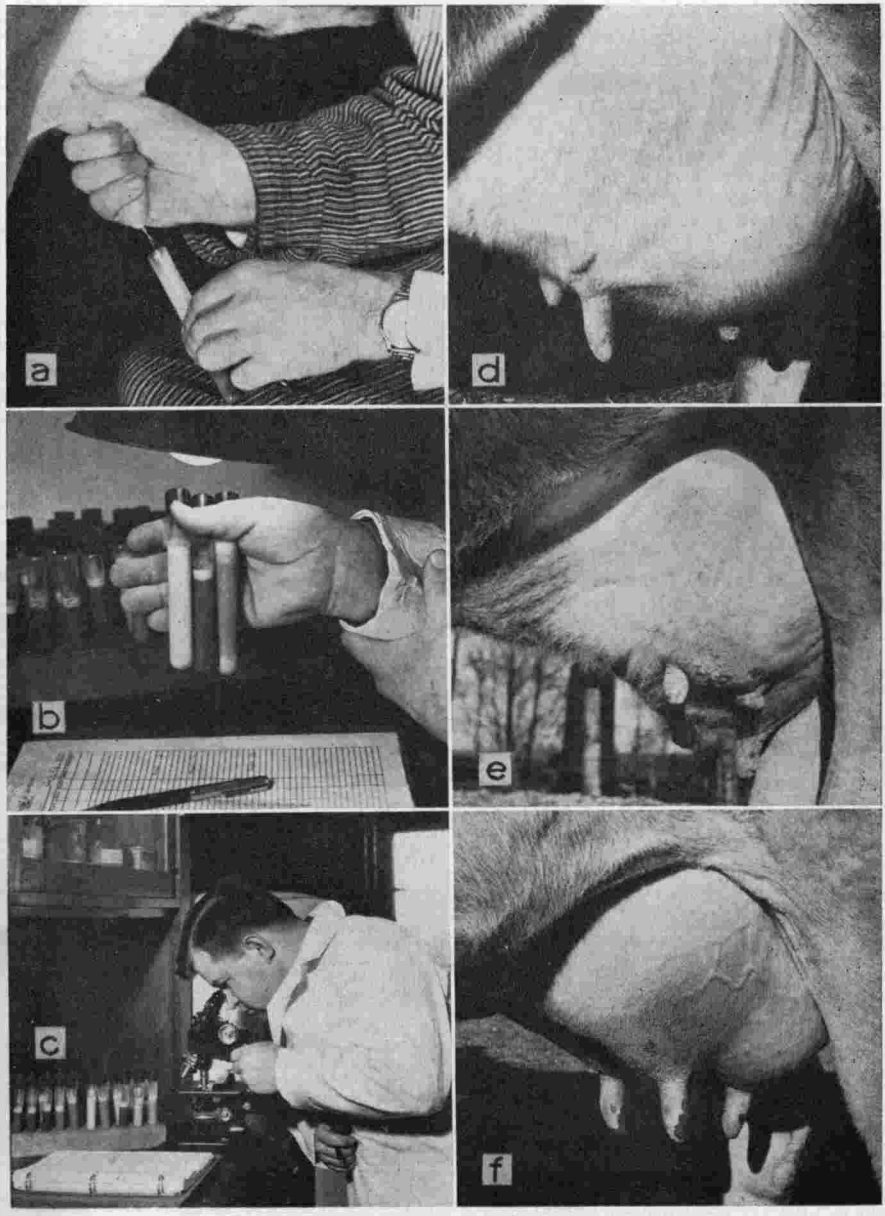

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COVER PICTURE

a. Collecting a sample of milk for the detection of mastitis-producing bacteria.

b. Reading the Hotis test for mastitis. The center tube is negative; the two outer tubes show varying degrees of a positive reaction.

c. Examining a stained film of incubated milk with the high-power microscope for the presence of mastitis bacteria and leucocytes.

d. A mastitis "flare-up" in a cow with the chronic form of the disease, causing a painful inflammation of the rear quarter, accompanied by thick, stringy, or watery milk.

e. The milk-secreting tissue in the udder of this cow has been destroyed by repeated attacks of mastitis.

f. The normal udder of a cow that has not been infected with mastitis; quarters uniform, pliable, and free from lumps.

MASTITIS STUDIES IN TENNESSEE

THE MASTITIS SITUATION IN TENNESSEE DAIRY HERDS AND INVESTIGATIONS RELATIVE TO ITS CONTROL

INTRODUCTION

Mastitis is a major problem of the dairy industry throughout the world. Various drugs and methods for treatment and control have been widely publicized and used by dairymen, but no satisfactory and sure method of control has yet been discovered.

More recently substances of pronounced bacteriostatic or bactericidal activity which are relatively harmless to the udder have been introduced. Great improvements have been made in the packaging and preparation of these materials and in making them more convenient for farmers to use. It now seems possible for an effective therapeutic attack upon the infectious organisms responsible for causing mastitis.

The investigation reported here was prompted with the view of measuring the costs and effectiveness of the best recommended practices for mastitis control. Incidental to the main objective, reliable information was secured concerning the incidence of mastitis infection in specialized dairy herds, the extent to which spontaneous recovery from the infection may occur, the level of infection in different age groups, and miscellaneous factors important in the mastitis problem.

REVIEW

The subjects of mastitis diagnosis, treatment, control, physiological effects and bacteriological problems have been thoroughly reviewed in a symposium prepared by Little and Plastridge (8). A more recent review of the subject has been presented in the review section of the Journal of Dairy Research (2). Evidence cited in these reviews indicate that mastitis can be markedly reduced by control measures based upon laboratory testing of quarter-samples of milk followed by treatment, segregation, or disposal of infected cows. Furthermore, a number of herds have been able to eliminate mastitis due to *Streptococcus agalactiae* completely and to remain free of these organisms for several years. These favorable results have prompted the establishment of statewide mastitis control programs in several states. A description of the Connecticut, New Jersey, Michigan and California programs is given in the sym-

posium of Little and Plastridge (8). In a report of the Illinois program, Alberts and Bryan (1) found 38.8 percent of tested cows infected with streptococcus, and the infection incidence was greatest in the more specialized dairy areas. Burkey, Buckner and Swett (5) reported that a program of continuous testing and treating was not effective in eliminating streptococcus mastitis from the dairy herds of the Bureau of Dairy Industry; and as the incidence of streptococcus infections declined, infections due to coliform and *Pseudomonas* species increased. Similar observations had been previously reported by workers with long continued experience in this field.

Most of the mastitis-producing organisms have been shown to be highly susceptible to the antibiotics *in vitro*. Packer (12) found that repeated treatment of infected udders with penicillin did not change the sensitivity of the organisms to penicillin. The failure of high concentrations of penicillin to overcome susceptible organisms in the udder may be due to lack of proper local concentration. Spencer and McNutt (19) described pathologic changes in infected udders which could account for poor penetration of penicillin into the affected area.

Penicillin has recently become the antibiotic of choice for intramammary infusion. In addition to its economy and proved efficiency against mastitis streptococci, penicillin has been shown by Schalm and Casselberry (15), Swanson and Herman (20), and Packer (12) not to interfere significantly with milk production. The amount of penicillin and infusion medium used in intramammary treatment has varied widely. Slanetz and Allen (17) found that three daily infusions of 20,000 Oxford units was as efficacious as three of 100,000 units, and that multiple infusions were better than single infusions. Schlam and Castleberry (15) recommended using 50 cc. of medium and 50,000 units of penicillin for multiple infusions. Packer (14) noted no difference in penicillin concentration in the milk following infusions of 25,000 to 100,000 units.

The persistence of mastitis infections greatly affects the mastitis control problem. The importance of resistance of the cow has been emphasized by reports by Breazeale and co-workers (3) and Merilan *et al.* (9). The former found that 11.4 percent of mastitis due to *S. agalacitae* cleared without treatment and 70.6 percent of the subclinical cases due to other streptococci cleared without treatment. The latter investigators reported that 38 percent of the untreated cows overcame the mastitis infection. Nearly all reports dealing with the mastitis problem emphasize the importance of

good herd management, which factor undoubtedly has a great effect through maintaining good health and resistance of the cows to harmful organisms.

EXPERIMENTAL METHODS AND MATERIALS

Detection of Mastitis Infection. — Since no one laboratory method has proved wholly adequate in detecting mastitis infection, a combination of three was employed in this investigation. The primary test was the Hotis incubation test (Hotis and Miller (7)). To the 0.05 percent bromcresol-purple solution used for tubes to be sent to the laboratory by mail or messenger, sodium azide (1:150,000) and brilliant green (1:500,000) were added as suggested by Bryan *et al.* (4) for inhibition of contaminating microorganisms. The Hotis tests were read after 20-24 hours incubation and again after 36-40 hours. In addition to the detection of streptococci as advocated by Hotis and Miller (7), the tests were carefully examined for evidence of staphylococci as directed by Schalm (16). Hotis tests were thus reported as positive for streptococci and for staphylococci.

Following reading of the Hotis tests, Breed smears were prepared from the incubated milk and microscopic examination made for the identification of the predominating types of microorganisms present. The average number of leucocytes found in not less than 10 fields was also recorded and the approximate number of leucocytes per milliliter was calculated.

Samples of foremilk were collected for the above tests, routinely, by the following procedure. The udder was washed in 400 ppm chlorine solution and dried. Two or three streams were drawn into a strip cup and evidence of abnormal milk noted. The teat orifice was then cleansed with a clean tuft of alcohol-soaked absorbent cotton. Milk was then drawn aseptically from each teat into a separate sterile test tube containing the Hotis test indicator. Evidence of abnormal milk was also recorded from the reaction color of the milk and the brom-cresol-purple.

Quarters were listed as infected if: (1) uncomplicated positive Hotis tests for streptococci were found; (2) incomplete or unusual Hotis tests for streptococci were noted and long chain streptococci were found on the microscopic examination; (3) staphylococci were indicated consistently (successive monthly tests) by the Hotis test and microscopic examination with or without many leucocytes; and (4) leucocytes in excess of 1,000,000 per milliliter were found ac-

companied by microorganisms.

Dairy Herds. — Dairy herds included in this study were three of the Experiment Stations plus three large herds operated by commercial dairymen in Knox county. These six herds were sampled on a monthly schedule beginning in December, 1947 and January, 1948. Other dairy herds from various parts of Tennessee were sampled only once or twice in order to measure the significance of the herds which were sampled more intensively. Ordinary herd management procedures were followed in all herds. All except one were milked by machine. Four different types or makes of milking machines were included. Sanitation during milking was generally acceptable. Two of the six herds sampled monthly used a four-pail washing-rinsing combine including separate wash cloths for each cow and a double rinsing of teat cups between cows. Prior to this investigation the herds had not been tested bacteriologically for mastitis and no strict milking order based upon mastitis history was followed. The prior intramammary treatment for mastitis in the various herds ranged from none to treatment of only a few acute cases. Therefore the initial mastitis incidence could be considered natural or normal in nearly all respects.

Plan of Investigation. The experimental treatments were conducted only in the six herds which were sampled at monthly intervals. The herds were divided arbitrarily into three groups of two each. One group was sampled at monthly intervals but no treatment given in order to determine the natural variations in mastitis infection which might occur. Another group was sampled at monthly intervals but no treatment was given any cows until they were dried off. The third group was sampled at monthly intervals, and following arrangement of the milking order from least infection to most seriously infected, treatments were made of all definitely infected quarters following each month's test. It was planned to make monthly tests until the mastitis infection was reduced to 5 percent or less for three consecutive months, when tests would be made only every 3 months. Incidence of infection was not reduced to the prescribed level; so monthly tests were continued throughout the entire observation period on all cows. The studies reported here cover a consecutive 24-month period in these six herds.

The herd owners or managers were informed of the results of each month's test currently and were advised to milk the infected cows separately or with a machine unit reserved only for this purpose. This advice was not strictly followed in all herds. Herd owners were specifically urged not to sell cows merely on the basis of the

bacteriological tests; since it was felt that this practice would have made the herd composition abnormal; and there was little assurance that it was an economical method of attacking the mastitis problem.

Treatments for Mastitis Infection. — In order to standardize the procedure, only one antibiotic was used for treatment. This was crystalline penicillin G as the sodium or potassium salt. Routinely the penicillin was dissolved in sterile distilled water just prior to use in such proportion as to give 1,000 Oxford units per milliliter. Then 50 milliliters (50,000 units) were injected into the gland cistern, using a sterile syringe and separate sterile teat cannula for each quarter. Four injections were made at 24-hour intervals as one course of treatment.

Variations of this treatment method were used in some phases of the investigation in order to check their effectiveness against the routine procedure. These variations will be presented in a discussion of the results from the respective comparisons.

The diagnosis of mastitis infection as used in this report is entirely bacteriological. Caution should be expressed concerning the evaluation of these results in terms of clinical mastitis. Since clinical mastitis is most often merely the culmination of an infectious condition that has existed for a lesser or greater period of time, it is not a proper base for experimental work of this nature. Farmers and veterinarians without access to proper laboratories can detect only clinical mastitis. Any sub-clinical mastitis diagnosis without laboratory aid is extremely unreliable. Likewise, the identification of the causative organisms without careful bacteriological tests is next to impossible. For this reason the levels of mastitis infection reported here may seem higher than practice has indicated.

OBSERVATIONS AND EXPERIMENTAL RESULTS

Initial Tests. — The results of the first test in herds that had not been treated for mastitis are presented in table 1. Herds A, B, C, D, E, and F are the ones which were subsequently sampled at monthly intervals, so an average of these herds has been compared with the average of all herds. All herds averaged 45.5 percent infected cows, while herds A - F averaged 49.1 percent infected cows. Thus the herds which were studied intensively were very similar to all specialized dairy herds sampled. These herds averaged slightly larger in size and had slightly greater initial infection than the average. The percentage of infected cows by herds varied from 21.4

Table 1.—Incidence of Mastitis Infection in Twelve Specialized Dairy Herds at Time of First Tests

Herd	Cows Tested	Cows Infected	Quarters Infected	Cows Infected	Quarters Per Infected Cow
	No.	No.	No.	Pct.	No.
A	39	26	63	66.7	2.4
B	57	23	48	40.4	2.1
C	16	4	8	25.0	2.0
D	30	20	45	66.7	2.3
E	64	32	68	50.0	2.1
F	14	3	7	21.4	2.3
G	25	13	19	52.0	1.5
H	30	14	35	46.7	2.5
I	34	13	19	38.2	1.5
J	19	11	21	57.9	2.0
K	21	7	8	33.3	1.1
L	38	10	15	26.3	1.5
All Herds Totals	387	176	356		
Av.	32.3	14.7	29.7	45.5	2.0
Six Herds A - F Totals	220	108	239		
Av.	36.7	18	39.8	49.1	2.2

to 66.7. In general, herds having a high percentage of infected cows also had a high number of infected quarters per infected cow, but this relationship was not significant. Two herds with less than average percentage of infected cows had a higher than average number of infected quarters per cow.

Extended Monthly Tests. — Although the data from table 1 indicate widespread existence of mastitis infection in Tennessee dairy herds, the true situation in a herd is usually worse, rather than better than that indicated by one test. Dry cows that have had one or more lactations have a better than even chance of being infected. In addition, more of the non-infected cows will appear infected in subsequent tests than will infected cows appear clear. Data from the first 12-month's tests in herds A - F are presented in table 2 to show the cumulative percentage of cows which showed

infection during the year. Treatment procedures were considered to have altered this picture only slightly, if any, during the first year. It is shown here that the total percentage of infected cows in these herds was 72.8, or nearly three-fourths of the cows. It is also evident from these data that the first test for each herd (table 1) is not a reliable index of the total yearly infection. Infection incidence in one herd (D) actually decreased when comparing the entire herd for the year with the first test. The herd (F) with the lowest initial infection incidence ended the year almost as high as the herd (A) with the greatest initial and yearly infection. A careful consideration of these data decisively eliminates any suggestion for controlling mastitis by selling all infected animals. It would be almost impossible to remain in the dairy business under such a plan. Some effective means of dealing with the infection in the farmer's present herds must be found and applied.

A more complete picture of the manner in which mastitis infection appears is presented in table 3. All the cows which were in the six monthly-tested herds for the full 24-month observation period were separated into groups according to the number of positive tests for each cow. It is noted from these data that only 6.7 percent of the cows remained clear of infection at every test,

Table 2.—Number of Cows and Quarters Infected with Mastitis in Six Herds During the First Twelve-Month Period.

Herd	Cows Tested	Cows Infected Sometime During Year	Quarters Infected During Year	Cows Infected	Infected Quarters per infected cow
	No.	No.	No.	Pct.	No.
A	57	50	155	87.7	3.1
B	90	61	155	67.8	2.5
C	41	24	69	58.5	2.9
D	40	26	70	65.0	2.7
E	80	61	177	76.3	2.9
F	20	17	47	85.0	2.8
Totals	328	239	673		
Av.	54.7	39.8	112.1	72.8	2.8

Table 3. — The Frequency of Appearance of Mastitis Infection in Cows Tested Monthly—(When Lactating) for Two Years

Infection		Cows	Cows
		No.	Pct.
Clear of mastitis infection all tests		14	6.7
Clear 1 or more months, infected for:	1 month	12	5.7
	2 months	17	8.1
	3 "	19	9.1
	4 "	11	5.3
	5 "	12	5.7
	6 "	18	8.6
	7 "	11	5.3
	8 "	5	2.4
	9 "	8	3.8
	10 "	8	3.8
	11 "	11	5.3
	12 "	6	2.9
	13 "	9	4.3
	14 "	7	3.3
	15 "	10	4.8
	16 "	8	3.8
	17 "	6	2.9
	18 "	4	1.9
	19 "	2	1.0
	20 "	5	2.4
Infected in 1 or more quarters every month		6	2.9
TOTALS		209	100.0

and likewise only 2.9 percent were infected at every test. The various treatments administered undoubtedly altered this situation from its natural course, yet the treatments were far from having a total protective effect. Without treatments the infection incidence should have been greater than it was. Yet, although an average of 51 percent of these cows tested clear on the first test only 20.5 percent were able to escape infection less than three months in two years. These data again emphasize the limitations of a single test, or even several successive tests. The wide-spread existence of mastitis infection and its persistence in spite of some type of treatment is also emphasized.

Age and Mastitis Infection. — Since Murphy (10) had postulated an age-susceptibility factor, and since this had been refuted by data presented by Ormsbee and Schalm (11) and Spencer and Kraft (18), it is of interest to present the incidence of mastitis according to age of the cow as found in this study. These data are pre-

Table 4. — The Occurrence of Mastitis Infection in Cows of Different Ages (Number of Lactations) Which Were Tested Monthly Throughout the Lactation

Number of Lactations	Cows Tested	Cows Infected	Cows Not Infected	Cows Infected
	No.	No.	No.	Pct.
1	116	39	77	34
2	94	63	31	67
3	76	49	27	64
4	67	50	17	75
5	68	49	19	72
6	60	50	10	83
7	50	39	11	78
8	27	24	3	89
9 and up	59	55	4	93
Unknown	47	38	9	81

sented in table 4 and percentage figures are graphically shown in figure 1. These were compiled by listing as infected any animal that exhibited one or more positive tests during any complete lactation. The number of lactations was known for the majority of the cows, and in others it was estimated from the age which was known. It was found that 34 percent of cows contracted mastitis infection sometime during their first lactation. In some herds a large proportion of these were infected at the first test following calving, but most of the heifers in all herds freshened clear of infection. The trend of infection is always upward with increasing age until after the ninth lactation nearly all of the cows tested were infected at some time in the lactation. The increase is not uniform, however The greatest increase occurred during the second lactation; and from the second to the fifth inclusive, little change in incidence of infection developed.

These indications are as much in support of frequent exposure causing mastitis as of age itself having a causative relationship. Certainly aged cows have been exposed much more frequently than young cows. Observation indicates that certain factors associated with age do, however, predispose to mastitis. For example, one cow, aged 15 years, was maintained clear of infection through the major part of two lactations when arthritis began to bother her severely. She soon developed definite mastitis infection (but not clinical mastitis) which persisted in spite of treatment until she was sold because of failure to breed. Other infirmities of the aged cows, including poor condition following calving, retained placenta and severely

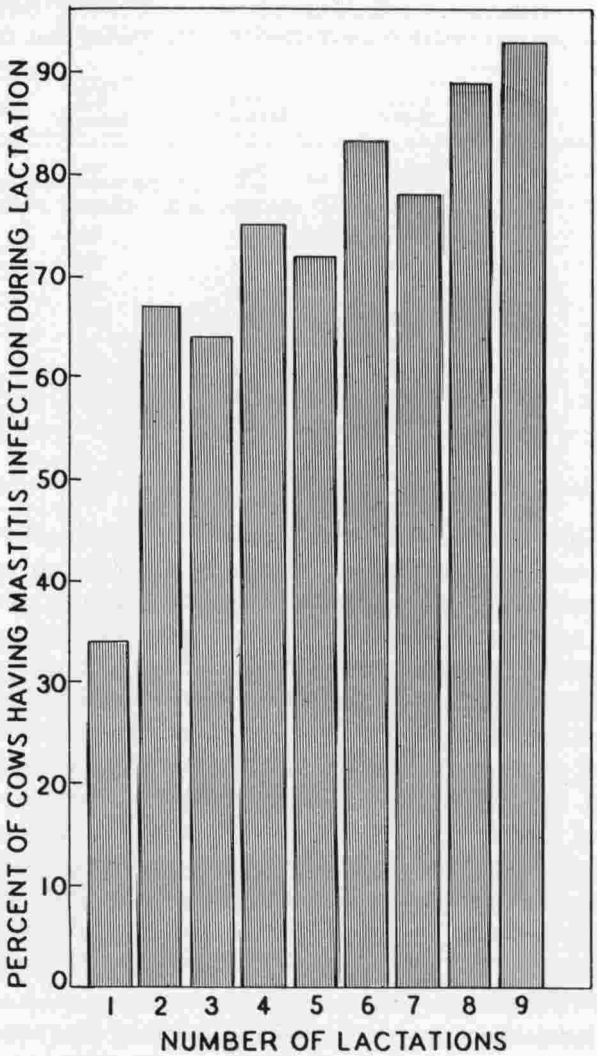

Fig. 1. The incidence of mastitis infection in cows according to age (number of lactations).

loosened udder attachments were also noticed to precede the first appearance of mastitis infection following many clear tests. The influence of general health to resistance to infections seems to be a very important factor in aged cows and appears to be a predisposing cause in mammary infections.

Infection Trends in Tested Herds. — The trend of mastitis infection in each of the six herds tested each month is indicated

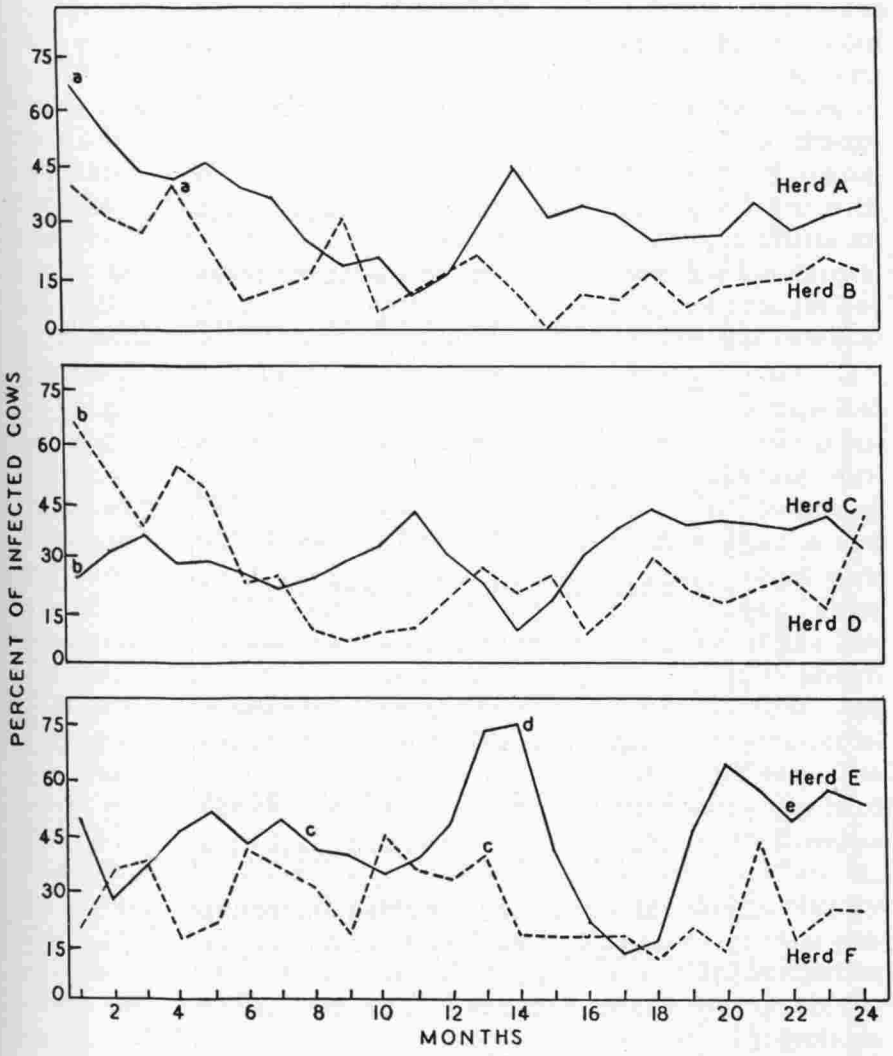

Fig. 2. Percent of Cows Infected with Mastitis-causing organisms at Monthly tests in Six Specialized Dairy Hards.

a. All positive quarters were treated each month following this test with quadruple infusions.

b. Infected cows treated only after drying off.

c. Infected cows treated with single penicillin infusions following this test.

d. Half herd treated each month with quadruple infusions of penicillin and half with single infusions following this test.

e. All treatments were stopped following this test.

Table 5. — Monthly Bacteriological Tests for Mastitis Infection in Six Specialized Dairy Herds in Tennessee

Month	HERD A				HERD B				HERD C				HERD D				HERD E				HERD F				TOTALS		
	Test. Cows	Pos. Cows		Pos. Qtrs.	Test. Cows	Pos. Cows		Pos. Qtrs.	Test. Cows	Pos. Cows		Pos. Qtrs.	Test. Cows	Pos. Cows		Pos. Qtrs.	Test. Cows	Pos. Cows		Pos. Qtrs.	Test. Cows	Pos. Cows		Pos. Qtrs.	Test. Cows	Pos. Cows		Pos. Qtrs.
	No.	No.	Pct.	No.	No.	No.	Pct.	No.	No.	No.	Pct.	No.	No.	No.	Pct.	No.	No.	No.	Pct.	No.	No.	No.	Pct.	No.	No.	No.	Pct.	No.
1	39	26	67	63	57	23	40	48	16	4	25	8	30	20	67	45	64	32	50	68	14	3	21	7	220	108	49	239
2	37	20	54	34	51	18	32	43	22	7	32	10	26	14	54	19	68	19	28	37	14	5	36	8	224	83	37	151
3	39	17	44	34	57	16	28	40	25	9	36	15	28	11	39	19	71	26	37	54	16	6	38	7	236	85	36	169
4	41	17	41	40	48	19	40	40	35	10	29	26	22	12	55	22	68	31	46	57	18	3	17	3	232	92	40	188
5	41	19	46	41	49	12	24	20	35	10	29	18	23	11	48	20	64	33	52	64	18	4	22	7	230	89	39	170
6	41	16	39	23	45	4	9	9	34	9	26	16	21	5	24	7	61	26	43	57	19	8	42	14	221	68	31	126
7	39	14	36	21	51	6	12	12	39	8	21	18	20	5	25	10	57	28	49	63	19	7	37	16	225	68	30	140
8	44	11	25	24	52	8	15	9	38	9	24	18	27	3	11	3	46	19	41	50	19	6	32	11	226	56	25	115
9	44	8	18	13	51	16	31	23	31	9	29	18	28	2	7	2	45	18	40	41	16	3	19	4	215	56	26	101
10	41	8	20	10	49	3	6	4	30	10	33	25	29	3	10	4	51	18	35	31	11	5	45	6	211	47	22	80
11	40	4	10	5	48	5	10	6	24	10	42	18	19	2	11	5	56	21	38	44	14	5	36	9	201	47	23	87
12	39	6	15	15	49	8	16	9	23	7	30	16	21	4	19	9	52	25	48	49	15	5	33	9	199	55	28	107
1st Yearly Totals	485	166		323	613	138		263	352	102		206	294	92		165	703	296		615	193	60		101	2640	854		1673
Av.	40	14	34	27	51	12	23	22	29	9	29	17	25	8	31	14	59	25	42	51	16	5	31	8	220	71	32	139
13	40	12	30	22	49	10	20	13	26	6	23	14	22	6	27	10	55	40	73	79	15	6	40	10	207	80	39	148
14	36	16	44	33	45	5	11	9	29	3	10	8	25	5	20	12	51	38	75	84	16	3	19	5	202	70	35	151
15	37	11	30	23	45	0	0	0	28	5	18	8	24	6	25	11	49	20	41	28	17	3	18	4	200	45	23	74
16	40	13	33	22	41	4	10	4	30	9	30	14	22	2	9	6	46	10	22	14	16	3	19	6	195	41	21	66
17	45	14	31	25	36	3	8	6	27	10	37	15	23	4	17	10	43	7	14	10	16	3	19	6*	190	41	22	72
18	45	11	24	14	34	5	15	7	28	12	43	19	23	7	30	12	42	7	17	11	15	2	13	2	187	44	24	65
19	41	10	24	17	35	2	6	2	32	12	38	19	24	5	21	7	41	19	46	30	14	3	21	4	187	51	27	79
20	40	10	25	19	38	4	11	6	31	12	39	19	33	6	18	11	45	29	64	60	14	2	14	2	201	63	21	117
21	39	13	33	28	38	5	13	8	29	11	38	17	28	6	21	8	46	26	57	55	16	7	44	8	196	68	35	124
22	46	12	26	25	43	6	14	11	27	9	37	12	21	5	24	8	44	21	48	38	18	3	17	7	199	56	28	101
23	46	14	30	30	37	7	19	12	25	10	40	14	19	3	16	5	46	27	59	55	16	4	25	5	189	65	33	121
24	50	16	32	29	38	6	16	8	25	8	32	16	20	8	40	14	52	28	54	62	16	4	25	5*	201	70	35	134
2nd Yearly Totals	505	152		287	479	57		86	337	107		175	284	63		114	560	272		526	189	43		64	2354	694		1252
Av.	42	13	30	23	40	5	12	7	28	9	32	15	24	5	22	10	47	23	49	44	16	4	23	5	196	58	29	104
Period Totals	990	318		610	1092	195		349	689	209		381	578	155		279	1263	568		1141	382	103		165	4994	1548		2925
Av.	41	13	32	25	46	8	18	15	29	9	30	16	24	6	27	12	53	24	45	48	15	4	27	7	208	65	31	122

* Previous month test used because of unreliable sampling.

graphically in figure 2. Data concerning the number of cows tested each month and number of cows and quarters infected are presented in table 5, along with period and herd averages. Rather wide variations in mastitis infection occurred in most of the herds. Some of the differences are explainable on the basis of treatments, change in milking methods, sale of cattle, or other items, but the reason for other differences has not been apparent. The experience with these herds indicates the improbability of eradicating mastitis infection by a testing and treatment procedure. Some of the important factors in the mastitis control problem will best be brought out by a more detailed examination of the experiments and observations in these six herds.

A procedure was adopted for two herds which was expected to result in the most rapid practical improvement in mastitis infection. The milking order in herds A and B was carefully controlled so that definitely infected cows were milked last. Following each monthly test, beginning with the first month in herd A and the fourth month in herd B the quarters which were infected were treated with 50,000 units penicillin daily for four days. Cows were treated both while lactating and after drying off, if the last test had been positive. The results of this regime are clearly shown in figure 2 and table 5. The percentage of infected cows was markedly reduced for 6 to 12 months, following which the infection seemed to plateau at a higher level than the minimum. The lowest percentage of infection was none in herd B and 10 in herd A, while the initial high level had been 40 and 67 percent respectively. During this time no infected cows were sold from herd A, but several from herd B were sold because of breeding difficulty or low production. It is evident that under this procedure the disappearance of the infection is only temporary or that reinfection occurs very readily. It was also noted that following an extended period of treatment in a herd the proportion of infections due to streptococci declined.

Treatment vs. Non-treatment. — A summary of the first twelve month's tests comparing the results in herds A and B with the untreated cows in herds C-F is presented in tables 6 and 7. In this study three consecutive clear monthly tests were required to constitute a cure. This is admittedly arbitrary and may be considered severe by investigators who commonly use only 1 or 2 weekly tests to indicate a cure. Experience, however, has indicated that failure to find the infection one or even two months following treatment cannot always be accepted as a cure because of

Table 6. — Summary of Elimination (Negative 3 consecutive months or more) of Mastitis Infectious Organisms from Quarters of Cows in Treated and Non-Treated Herds.

Herd	Quarters Infected	Quarters Reinfected	Quarters Cleared	Quarters Cleared	Treatment
	No.	No.	No.	Pct.	
A	144	23	136	81.4	Penicillin infusions
B	106	6	94	83.9	"
Totals	250	29	230	82.4	"
C	57	2	23	39.0	None
D	48	5	45	84.9	"
E	132	5	54	39.4	"
F	38	3	29	70.7	"
Totals	275	15	151	52.1	"

Table 7. — Summary of Elimination (Negative 3 consecutive months or more) of Mastitis Infectious Organisms from Udders of Cows in Treated and Non-Treated Herds.

Herd	Cows Infected	Cows Reinfected	Cows Udders Cleared	Cows Udders Cleared	Treatment
	No.	No.	No.	Pct.	
A	44	9	36	67.9	Penicillin infusions
B	40	2	38	90.5	"
Totals	84	11	74	77.9	"
C	25	0	7	28.0	None
D	20	2	16	72.7	"
E	42	0	10	23.8	"
F	13	0	5	38.5	"
Totals	100	2	38	37.3	"

the consistency with which the individual quarters must be classed "reinfected" under such a scheme. Such short time reinfections may just as well be considered reappearances of the original infection which was down but not out. Even using this severe a test of a cure, it is interesting to note that 52 percent of the quarters and 37 percent of the cows which were not treated subsequently were cleared of infection. Some of this improvement may have been due to rearranging milking order so as to reduce chances of exposure. Much of the improvement was definitely associated with improved milking practices. However, the resistance of the cow to the infectious organisms that invade the udder must be the major factor.

The aid of penicillin in eliminating mastitis infection is clearly shown in tables 6 and 7. About 30 percent more quarters were cleared than when penicillin was not used; but even more promising, over 40 percent more udders were entirely cleared of infection

with the aid of penicillin. It is also evident that penicillin treatment will rarely aid in complete elimination of the infection. In herd A, 32 percent of the infected cows were still infected, and in herd B about 10 percent did not respond fully to treatment. The penicillin treatments were definitely helpful but hardly equal to the glowing accounts found in the uninhibited advertisements of such material.

Efficiency of Treatment. Another indication of the efficiency of penicillin treatments can be seen from the data presented in table 8. These data were compiled from all of the quadruple treatments during lactation with 50,000 units of penicillin from which accurate evidence of cure could be gained. Some cows in herd E and herd G are included as well as cows in herds A and B. These data indicate the number of treatments required to effect a cure, and also the improbability of curing all cows or quarters, even with as many as 9 series of treatments. Sixteen cows were removed from the herds while still infected before as many as 9 treatments were given. These appear as uncured, and together with 3 cows not cured after 9 treatments, consist of 12 percent of all treated cows. The comparable percentage of uncured quarters was 8. Of the cows which were cured, only 60 percent followed one series of treatments, and only 67 percent of the cured quarters appeared following one series of treatments. Four series of treatments cured nearly all of the cows (97 percent) and quarters (96 percent) which were cured, yet at this stage infection remained in 12 percent of treated cows and 10 percent of treated quarters. In order to clear 403 quarters in these herds 787 quarter-treatment series were

Table 8. — The Effectiveness of Repeated Series of Quadruple Daily Treatments with 50,000 units Penicillin in Eliminating Mastitis Infection from Cows and Quarters.

Series of Treatments	Cows Treated	Cows Cured	Cows Cured	Cows Cured	Quarters Treated	Quarters Cured		
No.	No.	No.	Pct. treated	Pct. Cured	No.	No.	Pct. treated	Pct. Cured
1	158	83	53	60	438	270	62	67
2	73	27	37	19	164	78	48	19
3	42	16	38	12	75	34	45	8
4	25	8	32	6	38	9	24	2
5	15	1	7	.7	27	6	22	1
6	14	1	7	.7	18	1	6	.2
7	10	1	10	.7	14	3	21	.7
8	8	1	13	.7	10	1	10	.2
9	4	1	25	.7	3	1	33	.2
Totals	158	139	88		438	403	92	

given or nearly 2 series per cured quarter.

Stage of Lactation and Mastitis Infection. It has been observed commonly that clinical mastitis is more evident at the beginning and end of the lactation than at other times. Therefore, frequent recommendations have been given for mastitis treatment at the end of the lactation and during the dry period. At this time it is expected that the penicillin or other antibiotic will be diluted less with milk, and therefore more active, and also that treatment will be given during the period in which it may be needed most. The incidence of mastitis infection during each month of complete lactations in seven herds was tabulated to show the variation which normally occurs. These data are presented in table 9 according to four lactation intervals. The first and last intervals are each 2 months, and the remaining months of lactation were divided equally between the two middle intervals. Assuming a 10 month average for the lactations the percentage of mastitis infection has been weighted in the middle intervals to correspond to a 2 month period for comparison with the first and last periods. It is noted that there was little change in infection incidence during the first three periods, but a marked increase in the last two months of the lactation. The practice of treating cows during the dry period or just before drying off would therefore seem to attack mastitis infection at its highest incidence. If it is effective in controlling mastitis it would be the preferred method of treatment, also, because of less interference with milk supplies and less possible effect on lactation.

Table 9. — Influence of Stage of Lactation upon Relative Incidence of Mastitis Infection in Specialized Dairy Herds

Herd	Total Lactations During Which Infection Occurred	Stages of Lactation in Which Infection Appeared							
		1 - 2 Months		3 - 5 Months (orr 2d Qtr.)		6 - 8 Months (or 3rd Qtr.)		Last 2 Months	
		No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.
A	62	33	53	41	66	47	76	43	69
B	46	24	52	28	61	26	57	21	46
C	29	13	45	15	52	24	83	25	86
D	16	9	56	10	63	8	50	8	50
E	64	36	56	45	70	55	86	50	78
F	17	8	47	15	88	13	77	8	47
G	15	6	40	9	60	9	60	11	73
Totals	249	129	52	163	66	182	73	166	67
Weighted Average			52		44		49		67

Treatment During Drying Period. Herds C and D were tested monthly, followed by milking order adjustments in order to milk infected cows last or with a separate machine; and except for veterinary attention of cases of acute mastitis, treatments were given only during the dry period to infected cows. Infected quarters in herd C were treated with four daily injections of 50,000 units penicillin in distilled water, and herd D was given the same dosage in a mineral oil-water emulsion. There were no significant differences between the results in the two herds. Infection incidence in herd C increased during the year primarily because of increase in herd size through purchase of mature cows having some mastitis infection. Infection incidence in herd D decreased throughout the year. Two significant factors were responsible for part of this change. At the first test the milking machine was being improperly operated and the milker was not doing a careful job of milking. Repair of the milking machine, and later change in milking per-

Table 10. — Efficiency of Treatment with Penicillin Infusions During the Dry Period Only upon the Elimination of Mastitis Infection, and Comparisons

Item	No.	Pct.
Cows treated	27	
Quarters treated	71	
Treated Cows clear of infection 3 mos. after parturition	11	41
Treated Quarters clear of infection 3 mos. after parturition	45	63
Cleared cows reinfected before the end of the lactation	9	82
Cleared quarters reinfected before the end of the lactation	15	33
Clear cows not treated which freshened infected	8	29
Clear cows not treated which freshened clear	20	71
Infected cows not treated which freshened infected	11	92
Infected cows not treated which freshened clear	1	8

sonnel resulted in marked improvement in herd D. Then, late in the first year most of the infected cows were sold in a herd reduction.

Results from dry period treatments were slow to appear because of the relatively few treatments per month, and few cows which were milking each month which had just recently been treated. It was noted that reinfection frequently occurred in these cows so that it was necessary to retreat them at the following dry period even though a cure developed at the first treatment. The data in table 5 and figure 2 show that little consistent improvement in the mastitis situation in the herds developed when treatment was given only during the dry period. A summary of the efficiency of such treatments is presented in table 10. It will be noted from these data that 41 percent of the cows and 63 percent of the quarters which were treated during the dry period could be classified as cured in the first months of the next lactation. Reinfection claimed 82 percent of the cured cows; so the permanent effect of the dry period treatments was not significant. At the same time that treatments during the dry period were making little progress in the eradication of mastitis infection, 29 percent of the cows which dried off free of infection freshened with infection or contracted infection very soon after freshening. These factors combined were responsible for the slight change in mastitis infection in herds treated only during the dry period.

Although the dry-period treatment does not appear promising from these results, the situation may have been much worse without treatment. Only 8 percent of the cows which were infected when dried off, and were not treated, freshened clear of infection. This should be compared with 37 percent of non-treated infected cows which recovered during the lactation (table 7). This would indicate that infection persists more firmly in a dry than in a lactating udder, both with treatment and without treatment.

Staphylococcus infection was more prominent in herd C than in the others. Of the 18 cows in this herd treated during the dry period, 8 had staphylococcus infection either alone or mixed with streptococcus. A summary of the dry-period treatment in herd C, given in table 11, shows that these treatments were twice as effective against streptococcus infection as against staphylococcus. Identification of the causative organisms from acute mastitis just preceding or at parturition has frequently revealed staphylococci. In three months time three quarters on three cows were destroyed in this manner in one herd even though the cows were carefully treated with penicillin just after being dried off. This is another

Table 11. — Effectiveness of penicillin treatment during dry period in eliminating mastitis infection in Herd C.

Type of Infection	Cows	Infected Quarters					Infected Quarters Cleared		Infected Cows Cleared	
		When dried off	1st mo. lactation	2nd mo. lactation	3rd mo. lactation	4th mo. lactation				
		No.	No.	No.	No.	No.	No.	Pct.	No.	Pct.
Staphylococcus	2	11	4	5	6	4	5	45.5	0	0
Streptococcus	10	27	4	5	6	7	21	77.8	7	70.0
Mixed	6	5	4	4	4	4	1	20.0	2	33.3
Total	18	43	12	14	16	15	27	62.8	9	50.0

indication of the limitations of treatment in mastitis control.

No treatments were given to cows in herds E and F until the picture of mastitis infection changes without treatment had been well developed. It had been planned to study the effect of proper milking order and milking practice upon mastitis infection in these herds, but the frequent dairy personnel changes and other difficulties prevented the execution of this plan. The wide fluctuations in mastitis infection in these herds are probably quite typical, and are not greatly different from those of herds C and D, in which only dry-period treatment was given (fig. 2 and table 5). It is interesting to note that herd F was hand-milked, and yet had average mastitis infection, contrary to the frequently expressed idea that hand milking results in less mastitis than machine milking.

Comparisons of Treatment Methods. The cows in these herds and also of herd G were used to compare various methods of administering penicillin. A commonly used method of treatment employing single large infusions of penicillin was compared with four daily infusion treatments. Cows in the same herd were used for these comparisons. Part of the cows in the herd were given one intramammary treatment with 150,000 units penicillin in 60 cc. of distilled water. Other cows were given the multiple treatment as described above. The results of this comparison are summarized in table 12. The data presented there indicate that the quadruple treatment was more efficient in eliminating mastitis infection than was the single massive treatment. The difference on the basis of quarters cured is not large, but a higher percentage of cows (complete udders) were cured by using the four daily injections. Only 29 percent of the infected cows in these herds were cured by single injections as compared to 58 percent cured by quadruple injections.

Since the above data indicated that the efficiency of single treatments was low, it seemed important to test means of improving the single treatment procedure. It has been shown by Foley *et al.* (6) that penicillin in oil-water emulsions was retained in the mammary gland longer than penicillin in aqueous medium. Therefore a series of comparisons was conducted in one large herd in which all even numbered cows were given treatments with 150,000 units penicillin in 50 ml. distilled water, and odd numbered cows were treated with 100,000 units penicillin in 25 ml. of an oil-water emulsion.[1] The results of these treatments are summarized in table

[1] **Pencile** was the vehicle used for these trials. It was furnished by the Wallace Laboratories, Inc., New York, N. Y.

Table 12. — Comparison of Efficiency of Single Intramammary Injections of 150,000 Units Penicillin with Four Daily Injections of 50,000 Units Penicillin In Eliminating Mastitis Infection

Item		Single Treatment		Quadruple Treatment	
		No.	Pct.	No.	Pct.
Cows treated		65	100	30	100
Quarters treated		163	100	68	100
Cleared of Mastitis infection on 1st Mo. test	Cows	32	49	23	77
	Qtrs.	120	74	56	82
Clear of mastitis infection at 2d Mo. test	Cows	22	34	19	63
	Qtrs.	92	56	49	72
Clear of mastitis infection at 3rd Mo. test	Cows	19	29	16	53
	Qtrs.	78	48	37	54

Table 13. — Comparison of aqueous with oil-water emulsion vehicles for penicillin given in one intramammary injection

Item		Treatment 150,000 units penicillin in 50 cc water		Treatment with 100,000 units penicillin in 25 cc oil-water	
		No.	Pct.	No.	Pct.
Cows tested		21	100	19	100
Quarter treatments		65		68	
Quarters treated		49		45	
Cleared of mastitis infection on 1st mo. test	Cows	9	43	10	53
	Qtrs.	28	57	32	71
Clear of mastitis infection at 2d mo. test	Cows	7	33	8	42
	Qtrs.	21	43	25	56
Clear of mastitis infection at 3rd mo. test	Cows	4	19	7	37
	Qtrs.	18	37	23	51

13. The superiority of the oil-water emulsion as a medium for penicillin injection is apparent from these data.

Infectious Organisms Susceptible to Penicillin. After more than a year of treatment in herd A, it became apparent that some cows were either very slow to respond to treatment or very easily reinfected. One cow was treated in one or more quarters every month during two lactations without evidence of a permanent cure in any quarter. Others were not so extreme. The possibility of the infectious organisms developing or having resistance to penicillin was investigated. The infectious organisms from several of the quarters which had not responded to repeated treatments along with a few which did respond to later treatment were isolated by plating aseptically drawn milk on blood agar and subculturing typical colonies in tryptose broth. Organisms isolated from milk were maintained on tryptose-agar slants until tested. For the inoculum, 24-hour broth cultures were used. A drop of the inoculum was placed into tryptose broth containing varying concentrations of penicillin and incubated 48 hours at 37°C. Presence or absence of growth was recorded. All organisms grew well in the presence of 0.015 o. u. of penicillin per ml. Variations in growth occurred in cultures containing 0.031, or 0.062, and 0.125 o. u. of penicillin per ml. These results indicated no resistant strains and no marked difference between the strains tested.

DISCUSSION

The magnitude of the mastitis control problem is greater than most dairymen realize. When almost three-fourths of the milking cows in the herd shed infectious organisms sometime during the year, the most logical approach to the problem may be to consider every cow in the herd a possible source of infection at any time. Whether or not the infection progresses to the stage of clinical mastitis will depend upon the resistance of the cow to the infectious agent (s) and the care of the udder. The object of this investigation has been to determine the extent to which treatment is effective in this regard. The possibilities of mastitis control without antibiotic treatment and with various forms of antibiotic treatment in Tennessee herds have been explored.

The results indicate that even though the identity of the infected cows was known, the incidence of infection was not significantly lowered without treatment, except in unusual circumstances such as sale of cattle or changing seriously harmful milking prac-

tices. It is possible that infection incidence may have increased (as it did in herd E) if milking order according to the results of the tests had not been observed. The observation that 37.3 percent of untreated cows apparently recovered from mastitis infection would lead one to expect more favorable results without treatment than were secured. However, the incidence of new infections (original and reinfection) kept pace with the spontaneous cures; resulting in little change in average herd infection. A large percentage of the cows remained infected consistently from month to month, and many of them eventually developed acute or chronic clinical mastitis. A few cases of clinical mastitis developed in cows without previous positive bacteriological tests. These may have been due to invasion of the udder by the infectious agent after the last test, or to a rapid increase in the infectious agent from a level too low for detection to a level high enough to produce toxic changes. Such occurrences lead the farmer to lose faith in the ability of the bacteriological test to aid in the control of mastitis, because clinical mastitis is the only type which seriously concerns him.

Since the mastitis incidence was not lowered in herds which were tested but not treated, it might appear as if no benefit were derived, and therefore the cost of the testing was a complete loss in the total economic picture. Several benefits may accrue, however, from merely knowing which of the cows were infected and something about the seriousness of the infection. Milk for calf feeding can be selected from cows free of infection. This is an important item in reducing mastitis in heifers at their first parturition. When it is desirable to cull cows from the herd, knowing the degree of mastitis infection will aid the farmer to rid himself of the most serious sources of infection and at the same time salvage the animal by sale for beef before the disease renders it unfit for sale. There is also the possibility that knowing the infected cows may enable the farmer to partially protect the non-infected ones and thus increase the number of mastitis-free lactations and reduce the annual replacement cost due to mastitis. The nature of the data secured would not allow of an accurate estimate of the value of these factors.

To determine whether or not such testing would be economical it is necessary to estimate the cost of testing. On the basis of this study it has been estimated that while testing 4 herds averaging 25 cows each the average cost was $1.03 per cow, not including travel expenses. Since only one herd per day and four per week can be tested, the cost per cow will vary inversely as the size of

herds. Therefore large herds could more likely be interested in a testing program than could small herds. The experience in this investigation with samples collected by herdsmen and sent to the laboratory by mail was very unsatisfactory. In spite of preservatives, from 10 to 50 percent of such samples were unreadable. Therefore, the technician must be within daily driving distance of his laboratory—probably a radius of 100 miles per herd; this would then increase the average cost for a 25 cow herd to about $1.27 per cow. If four tests per year were made in a herd this would average $5.08 per average cow milking, or $127.00 per herd per year. In terms of milk value this is equivalent to 3175 pounds of milk at $4.00 per cwt. or 4233 pounds at $3.00 per cwt. Few farmers would be willing to pay so much for the seemingly intangible benefits which they would receive from such a testing program. This represents, however, less than the milk production lost from one cow having a severe acute attack of mastitis in early lactation, as well as less than the cost of raising one heifer for herd replacement.

Most farmers would more willingly provide money for drugs to treat their cows than for testing. Assuming an average cost of $1.00 per quarter treated, the $127.00 which might have been spent on testing would provide treatment material for 127 quarters or enough for nearly 32 cows per year. There may be some justification for regarding every quarter infected and treating the entire herd once or twice per year. Such a procedure certainly would reduce the infection in a herd at least temporarily and might provide the average farmer more direct benefit than a testing program alone. This would be a fruitful field for further investigation in mastitis control procedure.

An indication of what can be expected from treatments with penicillin in specialized dairy herds is given by the results from herds A, B, C and D. The cheapest form of treatment to follow would be treatment during the dry period, as practiced in herds C and D. It was not, however, outstanding in its effect, resulting in only 41 percent cured cows as compared to 53 percent of cows cured by one series of treatments during lactation. If a system of treating cows is planned it would be more efficient, therefore to treat all cows during the lactation than while they are dry. The failure of penicillin treatment during the dry period to produce as high a percentage of cures as that during lactation was somewhat unexpected in the light of previous reports. A logical explanation can be offered, however. It was frequently noted in this study that cows

sampled during the first week of lactation tested clear of infection but that infection appeared in subsequent tests in the same quarters which had been infected in the previous lactation. Hence, one clear test right after calving has not been accepted as evidence of a cure here as it has in many previous studies. On the basis of the known principles by which penicillin destroys bacteria, it would be expected that the penicillin would have greatest effect while the infectious organisms are able to multiply in the milk-bathed tissues of the udder, i.e. while lactating. During the dry period the udder is infiltrated with leucocytes and the composition of fluid in the milk ducts and cisterns becomes less favorable for bacterial multiplication, which is necessary for penicillin to destroy the organisms. It is also probable that the penetration of penicillin throughout the dry udder is not as great as in the lactating udder.

These investigations showed that mastitis was most rapidly and consistently reduced by the process of treating all positive quarters following each monthly test. This would then seem to be a desirable system to follow. The cost of such a plan might be considered prohibitive, however. The estimated cost of testing and treating herds A and B during the first 12 months was as follows:

Herd A			
485 cows tested	@ $1.27	=	$615.95
365 Quarters treated	@ $2.00	=	730.00
Total cost			$1345.95
Herd B			
613 cows tested	@ $1.27	=	$778.51
152 quarters (9 mos.) treated	@ $2.00	=	304.00
Total cost			$1082.51
Total both herds			$2428.46

Since a total of 144 cows were included in the program from these herds the first year, the average cost per cow was $16.86. If treathad been calculated at $1.00 (the cost of material) per quarter, the cost would still have averaged $13.27 per cow. It is also noteworthy that this program did not eliminate the mastitis infection so that it was necessary to continue treating in both herds the second year; therefore, it is not correct to assume that any large portion of the first year's cost could be prorated over a subsequent time period.

During the second year, most of the mastitis infection in herd A and some in herd B was due to a few cows which were periodic-

ally infected, cured, and reinfected or relapsed. In most cases these were high producing cows of fine inheritance; and although their elimination would have simplified the mastitis problem, it would not have been wise or an economical move in the long run. Furthermore clinical cases of mastitis did not develop more often in these cows than in others. In some of the clinical mastitis cases in this period no causative organism could be definitely identified, in others the infection was shown to be due to organisms which were noted very infrequently in the mastitis tests; such as *Aerobacter aerogenes, Pseudomonas* and *Corynebacterium.* It would thus be improper to assume that a herd with a very low incidence of detectable mastitis infection will be free of clinical mastitis or will cease to have quarters or cows destroyed by mastitis attacks. The chances for infection of the udder with a very wide variety of organisms which may be in its environment precludes the possibility of a complete eradication of the disease from an average herd of milk cows.

Summary

An investigation of mastitis infection as detectable bacteriologically in six typical Tennessee specialized dairy herds has indicated that an average of 72.8 percent of the cows in the herd are infected sometime during one year, but only 49.1 percent of the cows appear infected at one average test. Of all cows observed for a full two-year period, only 6.7 percent remained free of infection at every test. The incidence of infection in the cows almost doubled from the first to the second lactation and then remained high and slightly increased until 93 percent of cows with 9 or more lactations were infected. Spontaneous recovery from the infection occurred in 37.3 percent of infected cows. Treatment with penicillin aided in clearing the infection from many cows, but others did not respond to treatment. Organisms from some of the cows which were not cleared by repeated treatment were shown to be susceptible to penicillin *in vitro.* A series of four daily intramammary treatments with 50,000 oxford units of penicillin was more effective than single treatments with 150,000 units of penicillin. Of all cows which were cured following penicillin treatment, 97 percent were cured at or before the fourth series of quadruple treatments. Infection was found to be at its highest rate during the latter part of the lactation. Treatments during the dry period were not as effective as treatment during lactation in these herd-wide studies. The economy of a testing and treating procedure for mastitis control has been discussed.

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